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# The human $\alpha_1$-antitrypsin gene is transcribed from two different promoters in macrophages and hepatocytes

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**In order to investigate the mechanism of expression of the human $\alpha_1$-antitrypsin ($\alpha$1-AT) gene in macrophages, we have characterized the $\alpha$1-AT transcriptional units in these cells and discovered that there is a macrophage-specific promoter located ~2000 bp upstream of the hepatocyte-specific promoter. Transcription from the two $\alpha$1-AT promoters is mutually exclusive: the macrophage promoter is silent in hepatocytes and the hepatocyte promoter is silent in macrophages. In addition, in macrophages two distinct mRNAs are generated transcript by alternative splicing. These results suggest that $\alpha$1-AT gene transcription responds to two different cell-specific regulatory mechanisms.**

*Key words:* human $\alpha_1$-antitrypsin/gene expression/macrophages/promoter

## Introduction

Alpha-1-antitrypsin ($\alpha$1-AT) is one of the main protease inhibitors in human serum (for a review see Laurell and Jeppsson, 1975). It is believed that $\alpha$1-AT plays an important role in the control of the inflammatory response by inhibiting the excess elastase and collagenase released from leucocytes (Küppers and Black, 1974; Sharp, 1976). Single point mutations in the gene, leading to the synthesis of variant forms, are frequently associated with severe lung or liver disease (Carrell *et al.*, 1982; Kidd *et al.*, 1983; Nukiwa *et al.*, 1986). $\alpha$1-AT is synthesized mainly in the liver (Laurell and Jeppsson, 1975) and to a minor extent in macrophages (Perlmutter *et al.*, 1985a,b) and it has been shown that the same gene is responsible for $\alpha$1-AT production in both cell types (Perlmutter *et al.*, 1985b).

We have cloned the human $\alpha$1-AT gene and studied its expression into a variety of cultured human cell lines (Ciliberto *et al.*, 1985). We have shown that the 5′ flanking region of the $\alpha$1-AT gene contains sufficient information to drive accurate and hepatocyte-specific transcription of its own or of heterologous promoters (Ciliberto *et al.*, 1985). The hepatoma-specific information consists of several transcriptional signals each independently contributing to the rate and specificity of transcription (De Simone *et al.*, in preparation).

The $\alpha$1-AT gene is therefore expressed in a cell-specific manner. However, the fact that expression is found in cell types which originate from two different embryonal layers (hepatocytes from endoderm and macrophages from mesoderm) raises the question of whether a common mechanism for cell-specific expression in macrophages and hepatocytes exists, or whether the same coding sequence is transcribed in response to different regulators in the two cell types.

We have started, therefore, a detailed study of the macrophage $\alpha$1-AT transcriptional unit and found by a combination of cDNA cloning, S1 and primer elongation analyses that in these cells $\alpha$1-AT transcription starts from an upstream promoter. The overall structure of the macrophage-type $\alpha$1-AT mRNA is different from the hepatocyte-type $\alpha$1-AT mRNA for the presence of additional exons and the occurrence of alternative splicing.

The general relevance of such a complex pattern of differential tissue-specific gene expression is discussed.

## Results

### *Macrophage $\alpha$1-AT mRNA is larger than hepatocyte $\alpha$1-AT mRNA*

In humans, transcription from the $\alpha$1-AT gives rise in hepatocytes to the appearance of a 1400-base transcript with a 49-base-long 5′ untranslated sequence (Long *et al.*, 1984; Ciliberto *et al.*, 1985). The first 45 nucleotides derive from the transcription of a small first exon separated from the second exon by a 5-kb-long intron. In macrophages there is ~10-fold less $\alpha$1-AT mRNA (Perlmutter *et al.*, 1985a) but, as shown in Figure 1 (lanes M$\phi$1, M$\phi$2 and M$\phi$3), it is present in two distinct sizes (black arrows), both of them detectably larger than hepatocyte mRNA (open arrow). In our experiments we use as a source of uncontaminated hepatocyte mRNA, RNA extracted from the human hepatocyte cell line HepG2 (Knowles *et al.*, 1980). In most individuals the major macrophage transcript corresponds to the faster of the two bands; in some individuals, for instance M$\phi$1 shown in Figure 1, the slower and the faster species are present in approximately the same abundance.

### *Structure of the macrophage $\alpha$1-AT cDNA*

To precisely define the structure of the $\alpha$1-AT mRNA in macrophages, we constructed a cDNA library from human peripheral monocytes. Poly(A)$^+$ RNA from Ficoll-Hypaque-purified monocyte cells was used to construct a cDNA library in the vector $\lambda$-gt10. Plaques ($3 \times 10^4$) were screened using as probe the full length $\alpha$1-AT cDNA from liver. Thirty positive clones were obtained. Clones L17 and L25 which carried the longest inserts were subcloned and partially sequenced. The sequence of the 5′ part of these cDNAs is shown in Figure 2. Their comparison is very informative: they can be perfectly aligned for 207 bases from the 5′ end. Then there is an insert of 209 bases in L17, after which the two sequences can again be perfectly aligned for the remaining sequenced length which, starting from 57 bases after the end of the L17 insert, is identical to the liver-specific $\alpha$1-AT cDNA (Long *et al.*, 1984; Ciliberto *et al.*, 1985).

The 5′ portion of the macrophage $\alpha$1-AT cDNAs is homologous to different, non-contiguous genomic segments located upstream of the liver CAP site (Long *et al.*, 1984), thus showing the existence of additional exons (a schematic representation is shown in Figure 3). The upstream macrophage exon (exon A) is at least 207 bases long and contains the *Eco*RI site at position −1950 from the liver-specific cap site (Long *et al.*, 1984). The 101-nucleotide-long exon C, from position −56 to +45, is par-

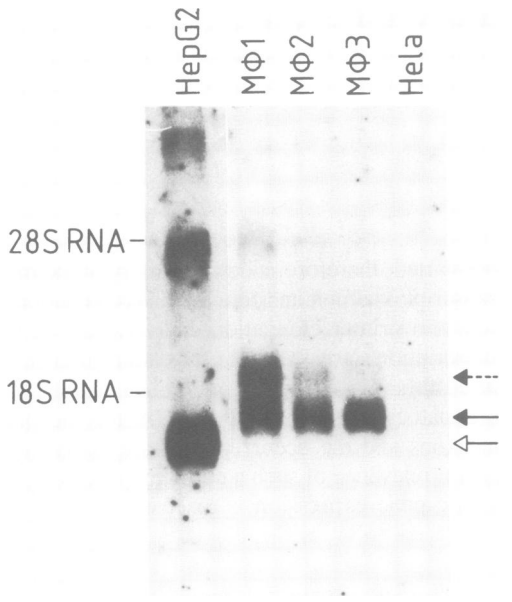


**Fig. 1.** Northern analysis of α1-AT mRNA from hepatocytes and from peripheral monocytes. Mϕ1, Mϕ2 and Mϕ3: RNA extracted from monocyte preparations from different donors. HepG2: RNA extracted from the human hepatoma cell line HepG2 (Knowles *et al.*, 1980). The open arrow points to the hepatocyte-specific mRNA, black arrows indicate the two different α1-AT mRNA species present in monocyte–macrophage preparations.

tially overlapping at the 3′ end with the first exon of the hepatocyte mRNA (exon C′, Figure 3). An additional 209-base-long exon (exon B) is therefore present in clone L17 corresponding to positions −401 to −192 relative to the liver-specific cap site. The difference between clone L17 and L25 reveals that there are at least two different α1-AT mRNAs, probably generated by alternative splicing of a common precursor RNA resulting in the inclusion or omission of exon B. This is in agreement with the detection of two α1-AT mRNA species in macrophages (Figure 1).

*Two different promoters are used in macrophages and hepatocytes*

We have investigated by S1 analysis whether the hepatocyte promoter is also active in macrophages and reciprocally whether the macrophage promoter is active in hepatocytes. To this end we used a 177-bp-long *Hin*fI–*Hin*fI segment spanning the liver start of transcription (from base −133 to base +44), shown in Figure 3. S1 protection of this fragment with RNA from human liver and from the human hepatoma cell line HepG2 (Knowles *et al.*, 1980) yields only a 44-base DNA species (Figure 4a), which is the length of the first exon in the hepatocyte α1-AT mRNA (Ciliberto *et al.*, 1985). RNA from peripheral monocytes protects only a 101-base-long segment corresponding to the length of exon C (Figures 2 and 3). The same 101-base-long protected DNA species is also present, although at a much lower intensity, in human liver RNA (Figure 4A, HL) presumably due to RNA originating from Kupffer cells which are macrophage-like cells characteristically present in liver sinusoids. This protected band is not observed when RNA from the hepatoma cell line HepG2 is used.

The position at which transcription starts from the α1-AT macrophage-specific promoter was determined by primer elongation and S1 mapping. For primer extension of RNA extracted from macrophages and from the hepatoma cell line HepG2, we used a 71-base-long DNA segment complementary to the sequence of both L7 and L25, from base 107 to base 177 (shown with a black bar in Figure 2). For S1 mapping we used a 5′ terminally labelled *Sma*I–*Eco*RI 299-bp-long segment (from position −185 to position +114 of the genomic sequence as shown in Figure 5 and schematically represented in Figure 3, upper part, P2).

The results of these experiments are shown in Figure 4. In panel B, showing the primer extension experiment, we observe three macrophage-specific products. The two longer ones, indicated by arrows, are primer elongated molecules of 184 (+113) and 147 (+76) nucleotides respectively. These correspond to RNAs which start at the equivalent positions predicted from the 114- and 77-base protected bands, revealed by the results of the S1 analysis of macrophage, but not hepatocyte, RNA shown in panel C. In panel B, there are additional bands, some of which are common to all lanes. No protected species corresponding to these RNA are seen in the S1 mapping experiment, and we therefore consider them likely to be due to non-specific hybridization of the primer used. The band indicated by a dot, which appears to be macrophage-specific but does not have a corresponding S1 protected band, is probably a consequence of the premature termination of reverse transcriptase. On the basis of the concordance between S1 mapping and primer elongation results, we conclude that there are two distinct macrophage-specific initiation points 37 bases apart. Their position on the genomic sequence is shown in Figure 5.

## Discussion

Like most plasma proteins, α1-AT is secreted from the liver into the serum. In the circulation it exerts an inhibitory action on plasma proteases. There are, however, situations in which a localized action is required. At the level of inflammatory or infectious foci there is accumulation of leucocytes and consequent release of relatively high amounts of proteases. α1-AT, secreted by intervening macrophages, prevents extensive tissue damage (Sharp *et al.*, 1976; Carrell *et al.*, 1982). In order to synthesize the same protein in two different cell types, two different, apparently mutually exclusive promoters, have evolved. In other cases, for instance in the α-amylase gene, which is expressed both in salivary glands and in liver, there is a salivary gland-specific promoter and another promoter active in both tissues (Shaw *et al.*, 1985). Macrophages and hepatocytes, however, are more distantly related during development than salivary glands and hepatocytes, and it is possible that *trans*-acting factors required for hepatocyte-specific transcription are not present in macrophages and vice versa. To prevent ubiquitous expression of tissue-specific genes, cells use specialized transcriptional systems. If, as is the case with α1-AT, the gene must be expressed in a subset of distantly related cell types, apparently a compromise cannot be found between the two specialized transcriptional apparatus and two different promoters are necessary. It will be interesting to find out if the same strategy is used in other similar cases, for instance for the transcription of the complement Factor B, also synthesized in hepatocytes and macrophages (Alper *et al.*, 1980; Colten *et al.*, 1979). In contrast, for genes that are expressed in all cell types, for instance those coding for the apoferritin subunits (Costanzo *et al.*, 1983, 1986; Santoro *et al.*, 1986), the same promoter is used in all cells, including macrophages and hepatocytes.

The two transcripts present in macrophages are probably generated by alternative splicing of a common precursor: the reason for this phenomenon is not known. One would imagine, also on

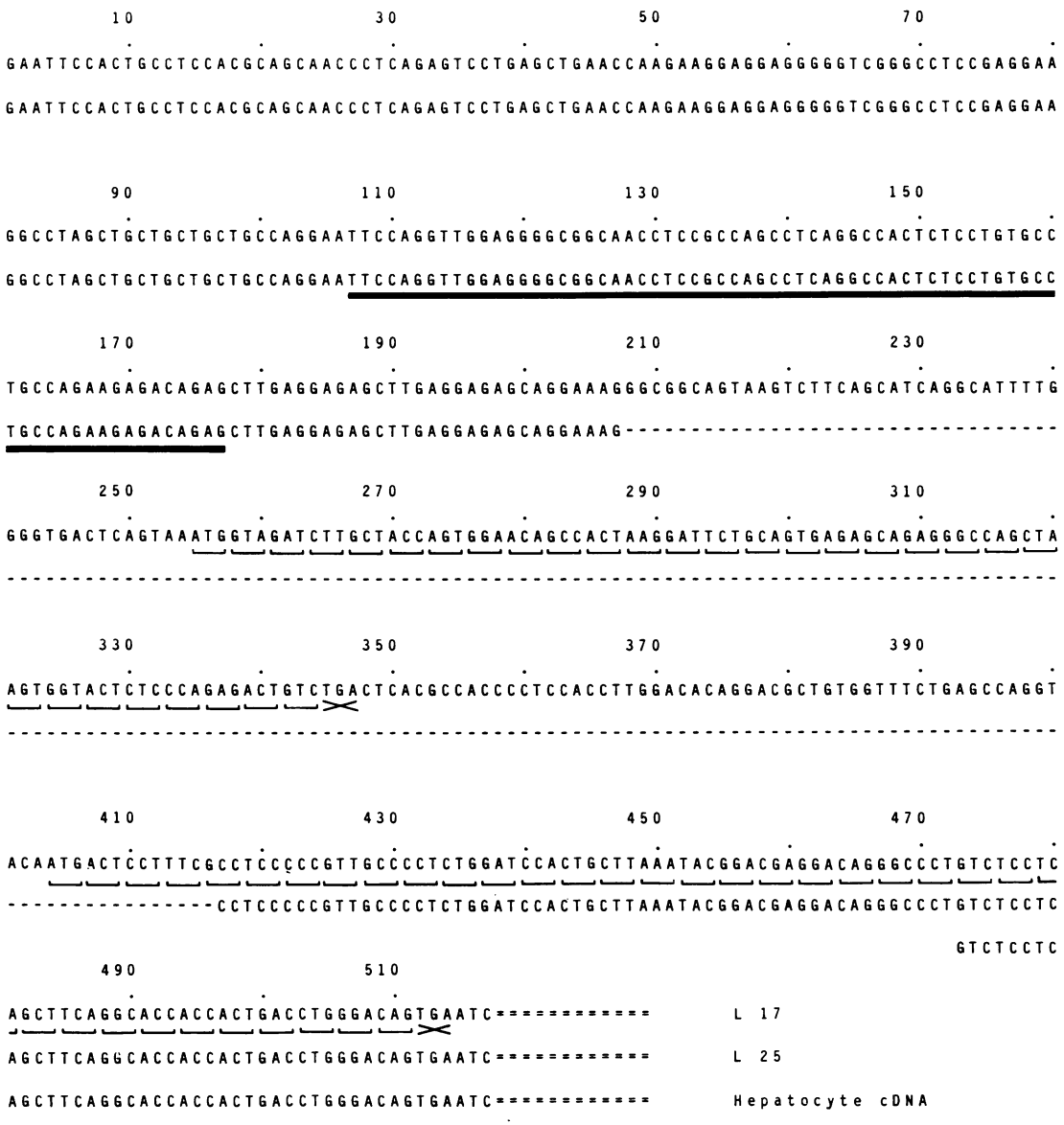


**Fig. 2.** Sequence of the 5′ end of macrophage α1-AT cDNAs. Sequence of the 5′ portion of the two different α1-AT macrophage cDNA clones L17 and L25 and their comparison with the 5′ end of the hepatocyte cDNA (bottom line) (Long *et al.*, 1984). The dotted line in L25 indicates the gap of 209 nucleotides in the structure of this clone with respect to L17 (see text). Codons defining open reading frames are indicated by brackets. The thick bar indicates the DNA segment used for primer elongation.

the basis of what has been observed in several other cases of alternative splicing (Ziff, 1980; Rogers *et al.*, 1980; Kornblihtt *et al.*, 1984; Tunnacliffe *et al.*, 1986; Leff *et al.*, 1987), that the two α1-AT mRNAs have different coding capacity. They code for an identical α1-AT molecule but the longer transcript shows two short open reading frames. One is contained within the exon B, the other begins towards the end of exon B and terminates in the third macrophage-specific exon (the first exon in the liver-specific transcript) (Figure 2). This observation is reminiscent of analogous situations observed in the 5′ flanking region upstream to the genes coding for the human and chicken oestrogen receptors (Green *et al.*, 1986; Krust *et al.*, 1986), the human transferrin receptor (Schneider *et al.*, 1985) or the yeast GcN4 regulatory protein (Mueller and Hinnebush, 1986). In this last case, convincing genetic evidence has been provided showing that the short upstream open reading frames are essential for the translational repression of GcN4 (Mueller and Hinnebush, 1986). In the case of the α1-AT the phenomenon of alternative splicing points to the importance of the information contained in the up-

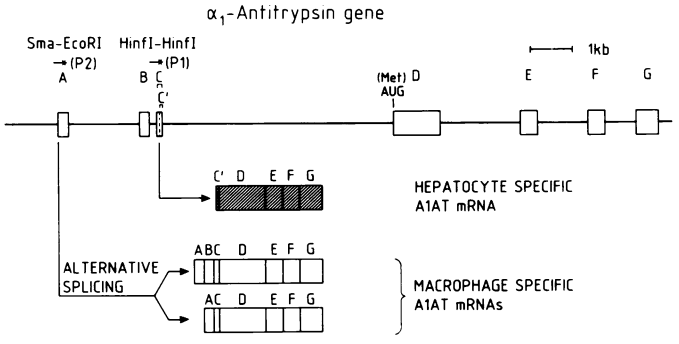


**Fig. 3.** Schematic representation of the α1-AT transcriptional units in hepatocytes and macrophages. The hepatocyte mRNA structure has been previously characterized by Long *et al.* (1984) and Ciliberto *et al.* (1985). The macrophage mRNAs structure has been deduced from the comparison between the sequences of cDNA clones L17 and L25 with the published genomic sequence of the human α1-AT gene (Long *et al.*, 1984). The ATG triplet coding for the first methionine of α1-AT is located in the common exon D. In the upper part of the diagram the two segments (P1 and P2) used as probes for the S1 protection experiments are indicated.

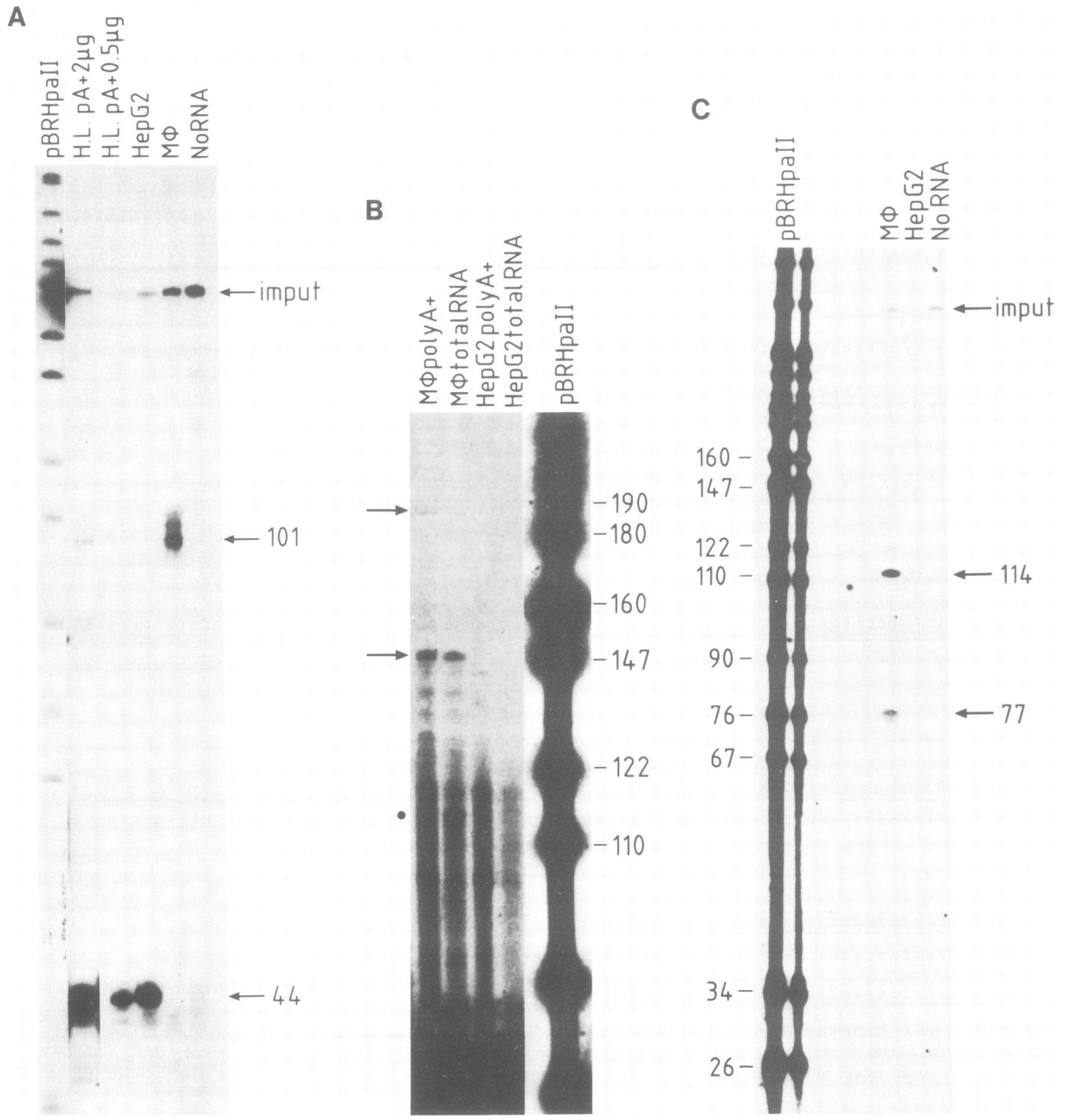


**Fig. 4.** The two α1-AT promoters act in a strict cell-specific manner. **(A)** S1 protection of a genomic *Hin*fI−*Hin*fI segment spanning the exons C−C′ (see Figure 3) with RNA from human liver, macrophages and HepG2. **B)** Primer elongation with an *Eco*RI−*Alu*I 71-base-long fragment complementary to the macrophage α1-AT cDNAs from base 107 to base 177 (Figure 2) on RNA from peripheral monocytes and from the hepatoma cell line HepG23. **(C)** S1 protection of a genomic *Sma*I−*Eco*RI fragment spanning the first exon of the macrophage α1-AT mRNAs by total RNA from monocytes and from HepG2.

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      -200                -180                -160                -140
       .                   .                   .                   .
AAAGGGCAGAGGGTGACTTGTCCCGGGTCACAGAGCTGAAAGGGCAGGTACAACAGGTGACATGCCGGGCTGTCTGAGTT

      -120                -100                 -80                 -60
       .                   .                   .                   .
TATGAGGGCCCAGTCTTGTGTCTGCCGGGCAATGAGCAAGGCTCCTTCCTGTCCAAGCTCCCCGCCCCTCCCCAGCCTAC

       -40                 -20                   1                  20
       .                   .                   .                   .
TGCCTCCACCCGAAGTCTACTTCCTGGGTGGGCAGGAACTGGGCACTGTGCCCAGGGCATGCACTGCCTCCACGCAGCAA

        40                  60                  80                 100
       .                   .                   .                   .
CCCTCAGAGTCCTGAGCTGAACCAAGAAGGAGGAGGGGGTCGGGCCTCCGAGGAAGGCCTAGCTGCTGCTGCTGCCAGGA

       120                 140                 160                 180
       .                   .                   .                   .
ATTCCAGGTTGGAGGGGCGGCAACCTCCGCCAGCCTCAGGCCACTCTCCTGTGCCTGCCAGAAGAGACAGAG
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**Fig. 5.** Sequence of the DNA region around the macrophage-specific α1-AT cap site. Boxed nucleotides are the two macrophage-specific transcriptional start points. Dotted boxes indicate a potential TATA-like sequence and an sp1 binding site.

stream open reading frames and suggests that it might be worthwhile examining this point further, especially in individuals like Mϕ1 in Figure 2, with unusually abundant α1-AT transcripts containing the exon B.

## Materials and methods

*Cell cultures, RNA extraction and Northern analysis*

Confluent monolayers of human macrophages were prepared from fresh blood from three different donors by adherence of Ficoll–Hypaque-purified mononuclear cells (Boyum, 1968). The human hepatoma cell line HepG2 (Knowles *et al.*, 1980) and the human carcinoma cell line HeLa B were cultured as previously described (D'Onofrio *et al.*, 1985). RNA was extracted with the guanidine-thiocyanate method (Chirgwin *et al.*, 1979), electrophoresed on 1.5% agarose–formaldehyde gel for 24 h at 30 V and transferred to nitocellulose filters as described (Costanzo *et al.*, 1983), 10 μg of total RNA being used for each lane. As probe we used the $^{32}$P-labelled (Feinberg *et al.*, 1984), 1400-bp human liver cDNA insert from clone pα1-5 (Chirgwin *et al.*, 1979).

*Construction and sequencing of a human monocyte–macrophage cDNA library*

Polyadenylated RNA was isolated by oligo(dT)–cellulose chromatography (Maniatis *et al.*, 1982). Double-stranded cDNA was synthesized as described (Glubey and Hoffman, 1983) starting from 2 μg of monocyte poly(A)$^+$ RNA. Cloning in the phage vector λgt10 was carried out following the procedure of Huynh *et al.* (1984). L17 and L25 *Eco*RI–*Eco*RI inserts were subcloned into the M13 TG131 vector (Kieny *et al.*, 1983) and sequenced with the dideoxy-chain termination method (Sanger *et al.*, 1977).

*S1 protection and primer elongation analysis*

The experimental conditions for S1 protection and primer elongation have been previously described (Berk and Sharp, 1977; Luse *et al.*, 1981; Ciliberto *et al.*, 1985). In each case $10^5$ d.p.m. of end-labelled fragment were used with 15 μg of total RNA or 0.5 or 2 μg of poly(A)$^+$ selected RNA from macrophages or HepG2 cells. After either S1 digestion (1000 U/ml, BRL) or primer elongation with AMV reverse transcriptase (15 U, Boehringer) samples were ethanol precipitated, resuspended in 2 μl formamide and loaded onto a 6% polyacrylamide, TBE 7 M urea gel and electrophoresed for 90 min at 2000 V. The gel was exposed for 3 days at 80°C with Kodak XAR-5 films.

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